

**Preliminary Amendment**

**Page 2 of 17**

Applicant(s): Olav K. LYNGBERG et al.

Serial No.: 09/647,475

Filed: 29 September 2000

Intl. Filing Date: 17 September 1999

For: COMPOSITE DEVICES INCORPORATING BIOLOGICAL MATERIAL AND METHODS

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Please replace the paragraph beginning at page 8, line 15, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

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a2 Other components (e.g., analytes in a sample of interest) that can be detected and/or quantitatively measured using the devices of the present invention include organic compounds that can be toxic to human, avian, plant, fish, insect, or other species. These include, for example, insecticides, herbicides, polycyclics, nerve gas agents, mutagens, carcinogens, antibiotics, products of combustion (e.g., tobacco smoke, coal combustion, liquid fuel combustion). Such compounds include hydrocarbons (e.g., xylene, toluene, naphthalene), halogenated hydrocarbons (e.g., trichloroethylene, carbon tetrachloride, chloroform), formaldehyde, ketones, hydrazines, and the like.

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**Page 3 of 17**

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Please replace the paragraph beginning at page 12, line 21, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

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a<sup>3</sup>  
Preferably, the biological material includes one or more species of prokaryotic, eukaryotic, or archean organisms as homogeneous cell populations, mixtures of microorganisms, consortia, mixed-cultures, or unspeciatiated naturally occurring microbial populations with a defined characteristic. The biological material can include mammalian cells, blood cells, bacterial cells, avian cells, plant cells, insect cells, spores (e.g., *Bacillus subtilis*), phages (e.g., lambda bacteriophage), viruses (e.g., HIV, HTLV), etc. The biological material can be in the form of cell clumps or cell mats (i.e., a number of different cells living together in some sort of structure), for example. Examples of suitable cells include bacterial cells such as *E. coli*, *Bacillus*, and *Streptomyces*, *Thermotoga*, archean cells such as *Pyrococcus*, eukaryotic cells such as yeast and *Penicillium*, as well as plant cells. For certain preferred embodiments, the biological material includes bacterial, yeast, or fungal cells, which may optionally be recombinant.

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**Page 4 of 17**

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Please replace the paragraph beginning at page 25, line 16, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

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*af* **Section B.** Patches of immobilized *E.coli* HB101 harboring the pOS14 *mer-lux* constructs were analyzed individually for luciferase activity after exposure to HgCl<sub>2</sub> concentrations from 0.1 nM to 10,000 nM, at 0.1 nM HgCl<sub>2</sub>. Light induction was not significant compared to the control. Hg(II) concentrations at 1 nM and 10 nM induced luciferase activity after a 4-5 hour lag, and luciferase activity increased during the next 10 hours of the assay. At 100, 1,000 and 10,000 nM HgCl<sub>2</sub>, luciferase activity was evident after 2 hours of incubation and reached the maximum detectable ( $6 \times 10^6$  count of single photons per minute) after 5-8 hours.

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Page 5 of 17

Please replace the paragraph beginning at page 27, line 6, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

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**Preparing Templates and Masks.** A template is a pressure sensitive tape with sections cut out where the coating liquid is to contact the underlying coating or substrate. A template can be applied on top of a clean substrate or on an already coated substrate. Masks are single pieces of pressure sensitive tape placed on top of the substrate or on coatings. After application of one or more layers on top of a template or mask each template or mask can be removed to expose the layer(s) beneath. Each template or mask was generated by manually punching with a 1/2 inch diameter punch (O'Brien Consolidated Industries, Lewiston, ME) or cutting with a razor blade. Templates were generated by taping 5 pieces of pressure sensitive tape (clear vinyl) to the backside of a template figure and each section was cut through the 5 template pieces simultaneously on a poly-vinyl chloride board. The individual template sheets were separated and cleaned with KIMWIPES to remove dust. Templates with nicks or tears were discarded since these would prevent them from separating from the substrate without tearing. Templates were applied onto the substrate or coating by rolling them onto it with a hard rubber roller (Orcon Corporation, Union City, CA). This method created uniform sections for patches or channels with a depth of 42.6  $\mu\text{m}$ .

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Page 6 of 17

Please replace the paragraph beginning at page 28, line 7, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

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*Ab*

**Latex Biosensor Patch Shown in Figure 14.** This device was created by coating a cell-latex mixture onto an 8 hole ½ inch diameter template on a polyester substrate. The coating was then dried. A sealant coating was coated on top of the cell coating with the template still in place. Following drying of the sealant coating the template was removed leaving two layered patches of approximately 60 micron thickness on the substrate. A mask consisting of ½ inch circles were applied to each patch. A spacer was laid around the patches on each side to prevent contact between the Mayer rod and the masks during coating. A nonporous coating was subsequently coated on top of the masked patches and dried before removal of the masks. Induction at 100 nM Hg<sup>2+</sup> resulted in a photon emission count of 500,000 counts per minute resulting from the mercury induced expression of luciferase. Induction at 0 nM Hg<sup>2+</sup> resulted in less than 61 photon counts per minute.

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Please replace the paragraph beginning at page 28, line 20, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

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a7 **Latex Biosensor Patch Shown In Figure 15.** This device was created by coating a cell-latex mixture onto an 8 hole ½ inch diameter template on a polyester substrate. The coating was then dried. An absorbent coating was coated on top and dried. A sealant coating was coated on top of the absorbent coating with the template still in place. Following drying of the sealant coating the template was removed leaving three layered patches of approximately 90 micron thickness on the substrate. A mask consisting of ½ inch circles were applied to each patch. Surrounding the patches on each side a spacer was laid down to prevent contact between the Mayer rod and the masks during coating. A nonporous coating was subsequently coated on top of the masked patches and dried before removal of the masks. Induction at 100 nM Hg<sup>2+</sup> resulted in a photon emission count of less than 1000 counts per minute resulting from the mercury induced expression of luciferase. The result demonstrated that the absorbent layer reduced the induced activity by 500 times. Induction at 0 nM Hg<sup>2+</sup> resulted in less than 50 photon counts per minute.

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**Preliminary Amendment**

Page 8 of 17

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Please replace the paragraph beginning at page 29, line 3, with the following rewritten paragraph. Per 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

**Latex Biosensor Patch Shown in Figure 16.** This device was created by coating a cell-latex mixture onto an 8 hole ½ inch diameter template on a polyester substrate. The coating was then dried. A sealant coating was coated on top of the cell coating with the template still in place. Following drying of the sealant coating the template was removed leaving two layered patches of approximately 60 micron thickness on the substrate. A second template consisting of ½ inch by 1 inch rectangular holes was applied on top of the patches so that 1/4 inch of the patches were covered and so that the open area connected to opposing patches. A porous channel layer was coated on top of the second template and dried. The second template was subsequently removed. A mask consisting of ½ inch circles was applied to each patch. A spacer was laid around the patches on each side to prevent contact between the Mayer rod and the masks during coating. A nonporous coating was subsequently coated on top of the masked patches and the porous channel layer and dried before removal of the masks. Each patch with its channel was subsequently excised so that each patch had a ½ inch channel attached. 5 mm of the channel end was placed in induction buffer, leaving the circular part with cells out of direct contact with the induction buffer. Induction at 100 nM Hg<sup>2+</sup> resulted in a photon emission count of than 50,000 counts per minute resulting from the mercury induced expression of luciferase. Induction at 0 nM Hg<sup>2+</sup> resulted in less than 110 photon counts per minute.